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LIVER DAMAGE PRODUCED BY FEEDING ALCOHOL OR SUGAR AND ITS PREVENTION BY CHOLINE*

BY

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[WITH PHOTOGRAVURE PLATE]

One of the first suggestions that over-indulgence in alcoholic beverages may damage the liver was made by Thomas Addison, physician to Guy's Hospital, London (Addison, 1836). He wrote: "With respect to the causes of this fatty degeneration of the liver, very little, or absolutely nothing, is known. In most of the cases which I have met with, there has been either positive or strong presumptive evidence that the individual had indulged in spirit-drinking; and indeed the most exquisite case I ever saw in a young subject occurred in a female who had for some time subsisted almost exclusively on ardent spirits. On the other hand, the extreme frequency of the degeneration in France, where the people are but little given to such indulgence, throws considerable doubt upon such an origin of the complaint."

A few years later Rokitansky (1849), pathologist to the Imperial Royal General Hospital in Vienna, reached a similar conclusion concerning the responsibility of alcohol for at least one type of fatty liver. Rokitansky noted the frequent occurrence of cirrhosis of the liver in drunkards. He was possibly the first to suggest that cirrhosis follows fatty changes in the liver.

The earliest references to experimental studies of the effects of alcohol were concerned with its effect on the brain (Dahlström, mentioned by Magnus Huss, 1852; Duchek, 1853). Kremiansky (1868), in the course of similar investigations, noted that one of four dogs receiving increasing doses of alcohol up to 6 oz. (170 ml.) daily for three months developed fatty changes of the liver and heart and a catarrhal condition of the kidneys. Ruge (1870), however, seems to have been the first to show clearly that an accumulation of fat occurs in the livers of dogs given increasing daily doses of alcohol by stomach-tube. He noted incidentally that the nature of the diet influenced the amount of fat found in the liver.

Moon (1934), in his comprehensive and critical review of the experimental work on alcoholic cirrhosis, concluded that none of the experimental procedures had been successful in producing cirrhosis in animals, other than rabbits, by alcohol alone. He pointed out that many of the so-called "positive" results with rabbits must be discounted owing to the presence of intercurrent liver infections (coccidiosis, etc.) or to a chronic periportal inflammation which is common among adult untreated rabbits. Moon concluded: "Without minimizing the contributory or predisposing influence which alcohol may exert, it must be concluded that experimental evidence has not substantiated the belief that alcohol is a direct cause of cirrhosis."

Experimental evidence that long-continued presence of abnormal amounts of fat in the liver does give rise to cirrhosis was supplied by Chaikoff, Connor, and Biskind (1938) using depancreatized dogs which had been maintained with insulin for periods up to five years. Production of cirrhosis in normal dogs by feeding alcohol to animals in which a fatty liver had previously been established by dietary means was reported by Connor and Chaikoff (1938), but the relationship of the alcohol to the cirrhosis became questionable when Chaikoff and Connor (1940) produced cirrhosis in normal dogs by dietary means alone (high fat and low protein). A series of papers from the National Institute of Health, Bethesda, Maryland, reported that the consumption of alcohol by rats increased the severity of dietary cirrhosis and that supplements of choline chloride or of methionine (or an increase in the dietary casein) prevented the development of cirrhosis and were beneficial in its treatment (Daft, Sebrell, and Lillie, 1941; Lillie, Daft, and Sebrell, 1941; Lowry, Daft, Sebrell, Ashburn, and Lillie, 1941; Lowry, Ashburn, Daft, and Sebrell, 1942; Lillie, Ashburn, Sebrell, Daft, and Lowry, 1942).

More recently, however, Ashworth (1947) concluded that alcohol exerts a toxic effect even when the diet is adequate. Rats on high-casein diets and others on low-casein diets were given 1.5 ml. of 25% alcohol per 100 g. body weight by stomach-tube daily for two months, and 10% alcohol was supplied in place of drinking-water; control groups without alcohol were "pair-fed" the same basal diets. Since all the rats lost weight, whether alcohol was present or not, it is questionable whether the basal diets were adequate. Interpretation of the data is therefore difficult (see "Discussion").

Although Ashworth (1947) pair-fed his rats, the calorie intake of those consuming alcohol was considerably in excess of those drinking water. To determine what effect calorie imbalance might have upon the results, we have performed experiments in which, for the first time in a

*A very brief account of this work was presented at the Detroit Meeting of the American Physiological Society, 1949.

study of the effects of alcohol, isocaloric pair-feeding has been used. Groups of rats not receiving alcohol were isocalorically pair-fed with those ingesting alcohol. The extra calories (equivalent to those supplied by the alcohol) were provided as finely powdered sucrose.

The effects of various lipotropic supplements added to the diets of the rats consuming alcohol were studied. The pathological changes in the liver produced in these experiments are attributed to an imbalance of calories and vitamins, particularly to an induced inadequacy of lipotropic factors consequent upon the increased calorie intake. Adequate amounts of choline chloride, methionine, or casein always protected the liver. Under these particular experimental conditions there is no more evidence of a toxic effect of pure ethyl alcohol upon the liver cells than there is of a poisonous action of an amount of sucrose which supplies the same calorie intake. In fact, there is no suggestion that either alcohol or sucrose exerts a direct hepatotoxic effect. The fatty and fibrotic changes are due to a deficiency of the lipotropic factors.

Experimental

A total of 188 male rats (150 to 200 g.) were divided among 12 groups unequal in numbers but with comparable weight distribution (see Table I). The animals were housed

TABLE I.—*Liver Lipids and Fibrosis*

Supplement	Diet Alone *ad lib.*	Diet *ad lib.* + Alcohol	Diet Alone (Pair-fed)	Diet Pair-fed + Sugar (Isocaloric)
None	**0** (10) 10% 0/6	**2** (20) 18% 7/15	**3** (32) 10% 2/28	**4** (12) 26% 7/9
Choline chloride 0·5%	**1** (6) 6% 0/6	**5** (20) 7% 0/18		**6** (12) 6% 0/12
Methionine 0·5%		**8** (20) 7% 0/18		**7** (12) 7% 0/10
Casein 17%	**9** (12) 7% 0/10	**10** (20) 8% 0/18		**11** (12) 7% 0/11

Average daily food consumption (without alcohol), 14 to 15 g.
Average daily food consumption (with alcohol), 11 g.
Alcohol supplied 18% total calories in Groups 2, 5, 8, and 10.
Group numbers in bold face type and original number of rats per group in parentheses. Average total liver lipids as percentage of fresh liver weight is shown below group number; number of livers showing fibrosis is given as numerator of a fraction in which denominator is the number of livers carefully examined after rats had eaten diets for 6 months.

in individual all-metal cages provided with a false bottom of coarse wire screen. A 15% (by volume) aqueous solution of purified ethyl alcohol was given in place of drinking-water to four groups of rats throughout the whole experimental period, which lasted 177 days. These groups (2, 5, 8, and 10) were offered their respective diets and the alcoholic solution *ad libitum*, and the amounts of each consumed were recorded daily. Four other groups of rats (4, 6, 7, and 11) started four days later were fed the same amount and kind of diet consumed by Groups 2, 5, 8, and 10, respectively. An attempt to achieve calorie equivalence was made by adding enough powdered sucrose to each of the former diets to supply the calories provided by the alcohol consumed by the corresponding group. These groups of rats and the remaining ones were given tap-water *ad libitum*. To assess the effects of the extra calories provided by the alcohol (or sucrose supplement) it is necessary to know the effects of the amount of basal diet consumed by the rats of Groups 2 and 4. The rats of Group 3 (started four days later than those of Group 2) were therefore "group pair-fed" on a diet-weight basis with those of Group 2. Only 12 rats were available for this group when the main experiment was started (June 24, 1948); 20 rats (referred to as Group 3A) were started one month later under identical dietary conditions. The rats of Group 0 were included to observe the effects of the basal diet alone when offered *ad libitum*, and those of Groups 1 and 9 were used to study the effects of the lipotropic supplements (choline and protein, respectively) when added to the basal diet and fed *ad libitum*. Further experimental conditions will be described in detail in the following paragraphs.

The Diet

A basal ration was desired which would be as nearly adequate as possible in all its dietary components except the lipotropic factors. However, it was considered inadvisable to permit an excessive accumulation of liver fat, since any effect attributable to alcohol alone would be difficult to assess if superimposed on a liver made excessively fatty by the basal diet. The small amount of choline chloride (0.05%) added to the basal ration was determined by preliminary experiments to be just sufficient to maintain the total liver lipids at about 10%, which was arbitrarily considered to be a satisfactory basal level for these particular studies.

The basal diet had the following composition, in percentages: casein 10, gelatin 5, zein 3, cystine 0.3, salts (Beveridge and Lucas, 1945) 5, celluflour 2, sucrose 41.7, starch 10, dextrin 10, beef fat 10, corn oil 2, "vitamin powder" 1, cod-liver-oil concentrate* 0.015, α-tocopherol acetate 0.01, choline chloride 0.05. The "vitamin powder" contained thiamin hydrochloride 0.5 g., riboflavin 0.25 g., pyridoxin hydrochloride 0.2 g., calcium pantothenate 1 g., nicotinic acid 1 g., folic acid 0.05 g., 2-methyl-1-4-naphthoquinone 0.1 g., para-amino benzoic acid 10 g., and inositol 50 g., made up with finely powdered sucrose to 1,000 g. Rats consuming 10 g. of diet daily received 50 μg. of thiamin hydrochloride and corresponding amounts of the other vitamins.

The methionine content of the diet was estimated to be 420 mg. and the cystine content (with the supplement) was 380 mg. per 100 g. Using calorie values per g. of 4 for protein, 4 for carbohydrate, and 9.3 for fat, the energy content of the basal diet was calculated to be 4.35 calories per g., of which 58% was contributed by the carbohydrate. The diets were freshly prepared at least once a week. The major dry ingredients for 5 kg. of diet were thoroughly mixed in a Hobart food-mixer, and a small portion (about 500 g.) was spread in a thin layer on a large tray. The minor ingredients (vitamin powder, cystine, or methionine) were ground individually in a mortar with small portions of the dry mixture, and this was then sifted (40-mesh sieve) over the surface of the material in the tray. The choline chloride was ground separately until uniform with about 10 g. of the dry mixture, and then more of the dry mixture was added to the mortar until about 40 g. was present. This mixture was sprinkled over the surface of the material in the tray. The cod-liver-oil concentrate and α-tocopherol acetate were dissolved in petroleum ether and sprayed over the same mixture. The contents of the tray were mixed by hand and then returned to the Hobart mixer and blended thoroughly. The beef fat was melted and brought to a temperature of 130° to 140° C., the corn oil was added, and the hot fat was poured all at once into the mixer and blended until uniform. The diets were kept in tightly closed tinned cans in a refrigerator at about 4° C. until required for use.

Alcoholic beverages and commercial ethyl alcohol contain compounds other than ethanol. To what extent the other components (in some cases unknown) contribute to the effects of alcohol upon the liver cannot be stated. Since

*This concentrate (Ayerst, McKenna, and Harrison, Ltd., Montreal, Canada) contained 200,000 i.u. vitamin A and 50,000 i.u. vitamin D per g.

the effects of ingesting alcohol may be due to the contaminants, the ethanol, or the ratio of dietary calories to the adequacy of other nutriments, it was considered important to eliminate the contaminants as completely as possible. Freshly prepared silver oxide (about 10 g. per litre) was added to so-called pure 95% ethyl alcohol. The mixture, shaken frequently, was left at room temperature for at least 24 hours. The alcohol was then decanted, made just alkaline with sodium hydroxide, and distilled through a packed fractionating column running at a reflux ratio of about 10 to 1. The first 20% of distillate was rejected, about 50% was collected, and the remainder was discarded. A 15% (by volume) aqueous solution of this material replaced the drinking-water of four groups (2, 5, 8, and 10) of rats. Fresh liquid was supplied twice a week. It was dispensed to the rats from 6-oz. (170-ml.) medicine bottles through a short piece of curved glass tubing with a tip slightly constricted in the flame so that liquid would not run out unless the rat lapped it. Sudden movements of the cage door were avoided to minimize leakage, since it was found that jarring the bottle permitted loss of a few drops of liquid. Losses due to evaporation were shown to be negligible (less than 0.3 ml. per day). Whenever, for any reason, abnormally large amounts of liquid seemed to have been consumed, leakage was suspected ; the average consumption of alcohol during the previous week was then used to calculate the calories thus obtained by the rat.

Technique of Feeding

The animals in Groups 2 and 4 were paid-fed on an isocaloric basis. The technique used in this isocaloric pair-feeding (which applies also to Groups 5 and 6, 8 and 7, 10 and 11) will be described in detail. The rats of Group 2 consumed the basal diet *ad libitum*, with 15% alcohol to drink ; the total calorie intake was determined from the average consumption of food and alcohol over three-day periods. The rats in Group 4 (started four days later) were offered the basal diet in an amount equal to the average value noted for those in Group 2, and enough finely powdered sucrose was added to this food (with thorough mixing) to supply the calories contained in the average amount of alcohol consumed by the rats of Group 2. The calculation of the amount of sucrose required was made on a somewhat arbitrary basis. Mitchell and Curzon (1940), in a review dealing with the food value of ethyl alcohol, stated that "for reasons at present unknown the energy of alcohol is not as well utilized in metabolism as is the energy of glucose." Mitchell (1935) estimated that the energy of alcohol is only about three-fourths as available for physiological purposes as that of sucrose. He stated that its growth-promoting power is definitely less than that produced by a sucrose supplement. In the absence of more specific information we assumed that calories from alcohol were only three-quarters as effective in causing gains in weight as were those of the sucrose supplements. Since 1 ml. of absolute ethyl alcohol weighs 0.79 g. and the calorie value is given as 7.1 cal. per g., then 1 ml. contains 5.6 cal., or 1 ml. of 95% alcohol corresponds to 5.3 cal. One ml. of a 15% by volume dilution of the 95% alcohol would supply 0.8 cal.: finally, if only three-quarters of this is available for promoting gains in weight, then 1 ml. is equivalent to 0.6 cal., or approximately to 0.15 g. of sucrose.

The rats were maintained on these dietary regimens for 177 days. Pairs of rats chosen at random from Groups 0, 2, 4, 5, 7, 8, 9, and 10 were killed at three weeks to determine to what extent deposition of liver lipids was taking place; again after 12 weeks pairs of rats from Groups 2 and 3 were sacrificed. All rats were anaesthetized with sodium amytal (90 mg. per kg. body weight, intraperitoneally). The animals were exsanguinated and the livers were removed and weighed immediately. Portions of right and left lobes were taken for histological examination ; the remainder was re-weighed and the total liver lipids were determined (Best, Lucas, Patterson, and Ridout, 1946). Blocks of liver, kidney, and certain other tissues were fixed in Heidenhain's SUSA mixture for 24 to 48 hours. Paraffin sections were stained with haematoxylin and eosin, and, to demonstrate connective tissue, with azocarmine, aniline blue, and orange G. The degree of fatty vacuolation of the liver cells was recorded as 0, +, ++, +++, or ++++. These histological data were in agreement with the results of chemical determinations, and therefore only the latter are included in the Table. Numerous sections from each liver were examined carefully for signs of fibrous-tissue proliferation.

Results

No abnormalities were detected on gross, microscopical, or chemical examination of the livers of any of the animals receiving the diets containing the lipotropic supplements, whether or not alcohol (or extra sugar, isocaloric with the alcohol) had been consumed. The rats maintained on the basal diet alone (Group 0) grew well and (with the exception of one rat which showed some pancreatic disease) the total liver lipids ranged from 7.7% to 12.8% with an average of 9.3%, and no liver fibrosis was observed. The average daily food consumption of the rats allowed to eat this diet *ad libitum* was reasonably constant at about 14 to 15 g., as was that of the other rats not consuming alcohol and eating *ad libitum* (Groups 1 and 9). All the rats consuming alcohol (Groups 2, 5, 8, and 10) ate less—only about 11 g. daily. The rats in the pair-fed groups (3, 4, 6, 7, and 11) were therefore offered 11 g. of the corresponding diets daily. The rats of Group 3 gained less weight because of this restricted food intake, but their livers showed essentially the same chemical and histological picture as those of the rats of Group 0.

The rats consuming alcohol without adequate lipotropic factors in the diet (Group 2) developed fatty livers with an average total lipid content (20%) about double that of the rats consuming the same amount of diet without alcohol (Group 3). One-half of the surviving rats drinking alcohol and eating the basal diet (Group 2) exhibited hepatic fibrosis, while in those without alcohol (Group 3) fibrosis could be detected in only two out of 28 survivors, and in one of these the fibrosis was minimal. The rats fed sugar isocalorically—i.e., those of Group 4—had on the average more fat (26%) in their livers and a somewhat higher incidence of fibrosis (7 out of 9 survivors) than those receiving the alcohol (Group 2). However, in none of the livers of either Group 2 or Group 4 had the fibrosis advanced sufficiently to be considered frank cirrhosis. The greater production of fibrosis in the rats of Group 4 (sucrose) than in those of Group 2 (alcohol) probably may be explained by our failure to achieve an exact equivalence in calories between the rats of these two groups. Some evidence for this is apparent in Chart I, in which it will be noted that the average final weight of the rats receiving the sugar supplement exceeded by about 16 g. that of the rats consuming alcohol. This is a rather small discrepancy in weight at the end of a test period of six months, but the difference it represents in calorie intake may be sufficient to account for the slightly greater liver damage. Chart II indicates a more successful attempt at pair-feeding in the rats receiving choline. The curves require no further comment except that the dips seen in all of

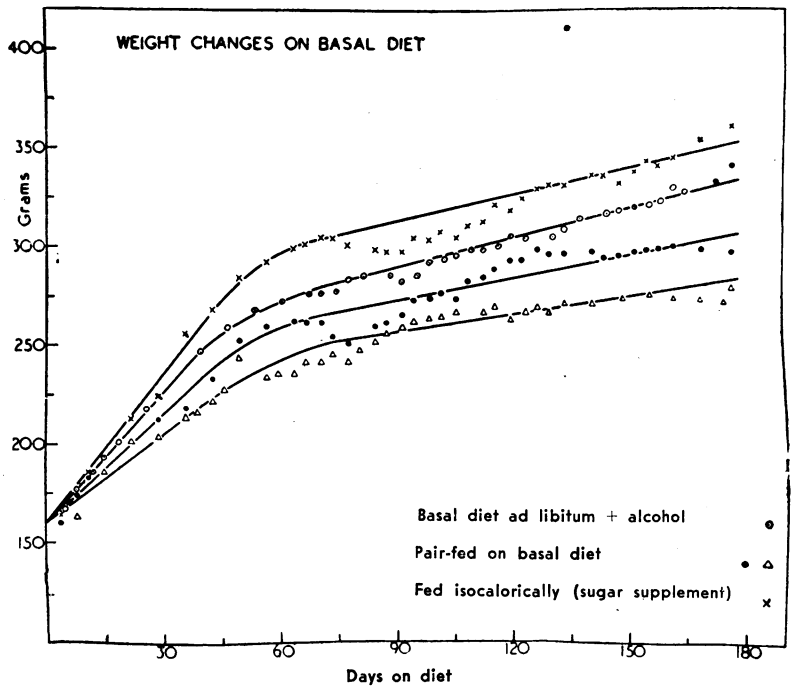

CHART I

them at about 80 to 90 days are related to a period of exceedingly hot weather, during which the air-conditioning equipment failed to function properly. Since the growth curves of the animals given supplements of methionine or casein (Groups 7, 8, 10, and 11) are essentially the same as those in Chart II, they will not be presented. The rats of Group 0 (basal diet *ad libitum*) grew most rapidly, those of Groups 1 and 9 only slightly less well. The rats of Group 0 consuming 14.9 g. of food per day (65 calories) gained on the average 262 g. Those of Group 3 consuming 11.2 g. (49 calories) of the same diet gained only 137 g. Rats of Group 2 eating essentially the same amount (11.4 g.) of diet but obtaining also 11 calories from alcohol—i.e., a total of 61 calories per day, 18% of which came from alcohol—gained 182 g. Rats of Group 4 ate 11.2 g. of diet daily and were given an average sugar supplement of 2.7 g. (total calories 60, of which 18% was supplied by the sucrose supplement); they gained 198 g. Thus rats of Group 0 eating 3.5 g. more food per day than those of Group 2

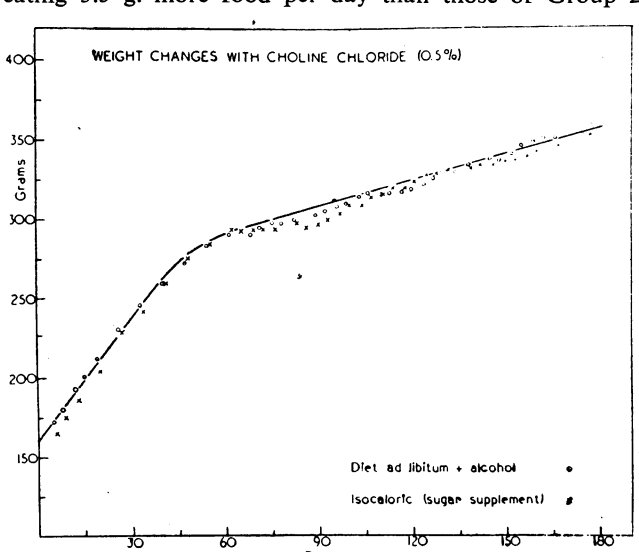

CHART II

gained 80 g. more than did the latter; in spite of the calories obtained by the rats of Group 2 from the alcohol, they were still getting from 4 to 5 calories per day less than those eating the basal diet *ad libitum*. The stunting of growth frequently attributed to the toxic action of alcohol is in these experiments due mainly to decreased food intake, unless one is willing to make the highly improbable assumption that sucrose exerts the same toxic effect as alcohol.

Fig. 1 (Plate) shows the typical histological picture seen in fatty livers (without fibrosis) of the rats consuming the basal diet with alcohol; Fig. 2 shows the same for rats on the basal diet with sugar fed isocalorically; Figs. 3 and 4 illustrate the distribution of fibrous tissue (when present) in the livers of the two groups. After careful study of many sections from all the livers these two examples were selected as representative of the two groups. The similarity in fat deposition and in the nature and extent of the fibrosis in the livers of the two groups is remarkable. Equally striking is the absence of abnormalities in the livers of the animals receiving adequate lipotropic supplements, as has already been mentioned.

Discussion

Clinicians in North America and Europe have tended for many years to associate chronic alcoholism and cirrhosis. In parts of Asia and Africa, however, cirrhosis is common although alcoholism is rare. In these regions it has been noted that cirrhosis occurs most often in persons subsisting on deficient diets. Thus a consideration of the incidence of cirrhosis throughout the world reveals a correlation with malnutrition. This situation may be obscured in some areas by a high incidence of cirrhotic changes following various forms of hepatitis. Within recent years it has been recognized that the chronic alcoholic, like other malnourished individuals, often shows signs of various dietary deficiencies: the intakes of good proteins and of vitamins of the B complex are generally low.

We are not considering in this paper the effects of alcohol on tissues other than the liver. Whether or not alcoholic beverages *per se* directly injure the liver has been a subject of controversy. Even if it could be proved that these beverages do exert a toxic effect upon the liver it would still have to be shown whether the effects were due to pure ethanol, to associated toxic contaminants, to a general malnutrition, or to one or more specific dietary deficiencies.

The present series of experiments does not supply any information whatever on the possible effects of toxic agents which may occur as natural contaminants of alcoholic beverages available in commerce. However, fatty infiltration and consequent fibrosis, which closely resemble the lesions in the chronic alcoholic, have been seen in these experimental animals consuming a purified alcohol. One is therefore tempted to assume that the hypothetical toxic contaminants may be of minor importance in causing the liver lesions seen in the human alcoholic.

The above experiments show that a carefully purified sample of ethanol can cause an excessive accumulation of fat in the liver and subsequent development of fibrosis when the diet lacks adequate amounts of the lipotropic factors. Since, however, pure sugar caused lesions of a similar nature and extent, and since these, as well as those due to alcohol, were entirely prevented by dietary choline or its

C. H. BEST, W. STANLEY HARTROFT, C. C. LUCAS, AND JESSIE H. RIDOUT: LIVER DAMAGE PRODUCED BY FEEDING ALCOHOL OR SUGAR, AND ITS PREVENTION BY CHOLINE

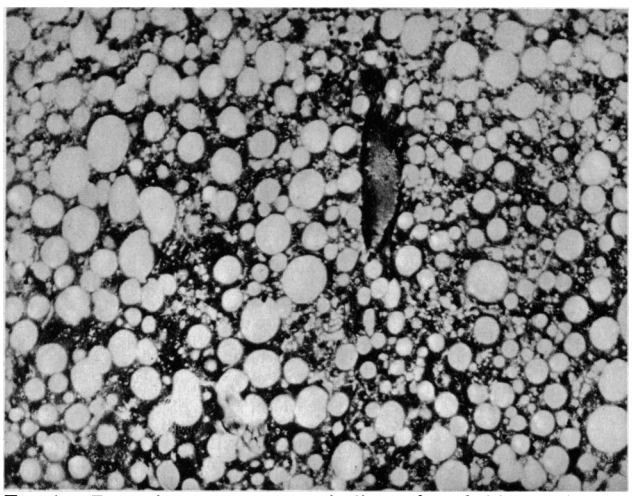

FIG. 1.—Fatty change (++++) in liver of rat fed basal diet and given 15% alcohol in place of drinking-water. Paraffin section; azocarmine, aniline blue, and orange G. (× 80.)

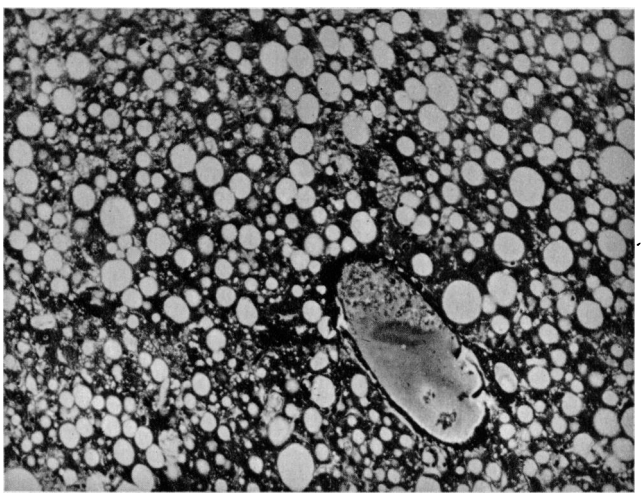

FIG. 2.—Fatty change (++++) in liver of rat fed basal diet and sugar. Paraffin section; stain as in Fig. 1. (× 80.)

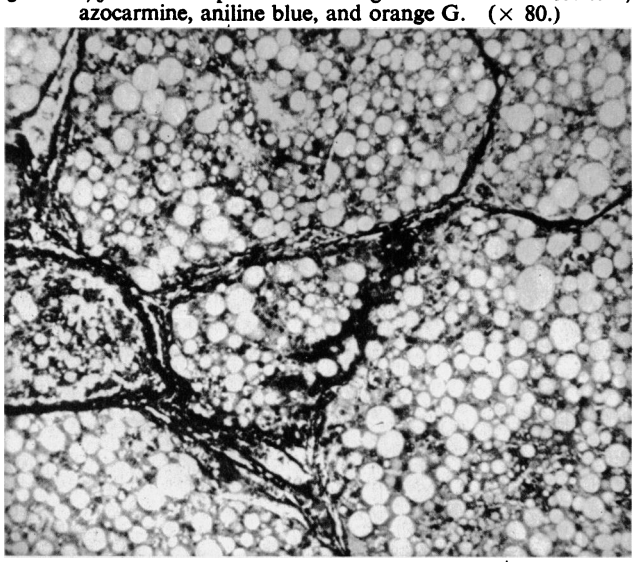

FIG. 3.—Well-defined fibrosis in liver of rat fed basal diet and given 15% alcohol in place of drinking-water. Paraffin section; stain as in Fig. 1. (× 80.)

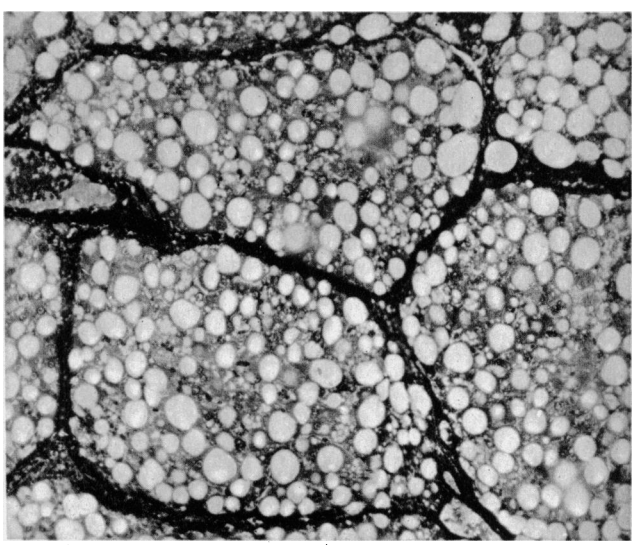

FIG. 4.—Well-defined fibrosis in liver of rat fed basal diet and sugar. Paraffin section; stain as in Fig. 1. (× 80.)

J. F. CURR: SYNOVIAL OSTEOCHONDROMATOSIS

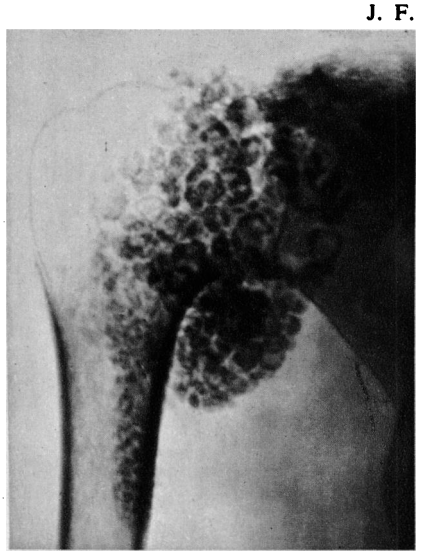

FIG. 1.—Case 1. Right shoulder: large numbers of osteochondromata.

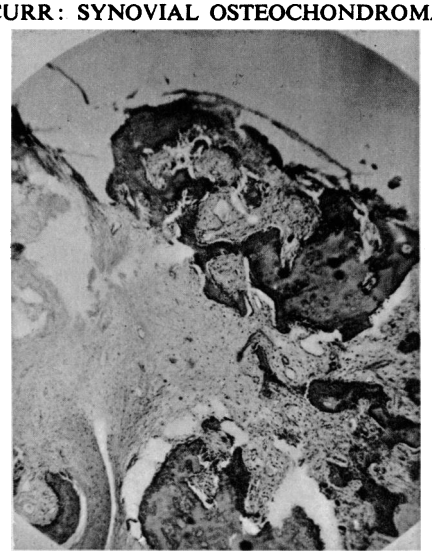

FIG. 2.—Case 1. New bone formation. (× 60.)

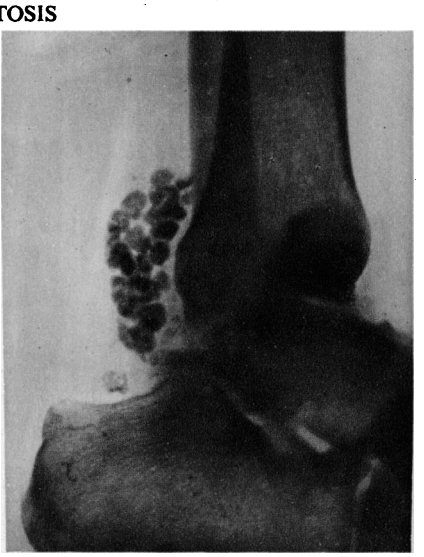

FIG. 3.—Case 2. Left ankle: osteochondromata posterior to joint.

precursors (methionine or casein), the idea of a specific toxic effect of alcohol upon the liver cells receives no support. While the effects of general malnutrition should not be minimized, the experimental evidence establishes the fact that a specific dietary deficiency—viz., of choline—is involved in the production of the liver damage which we have observed.

Many of the older experiments, as well as some of the more recent ones in which the role of alcohol in the aetiology of liver damage was studied, were poorly controlled. When dogs, cats, rats, or other small animals are given such large doses of alcohol by stomach-tube that they are in a comatose condition during most of the time, the food consumption is inevitably low. Stunting of growth or loss of weight could be thus explained, and possibly also certain types of liver damage. In some other studies that have been reported the experimental animals were offered diets which were inadequate in many respects. To what extent multiple dietary deficiencies have influenced the effects of the alcohol in these experiments cannot be determined. The diet used in our investigation has been designed to be adequate (in terms of present knowledge) with regard to all the essential amino-acids, all available vitamins, minerals, and essential fatty acids. In other words, the diet is believed to be satisfactory in all respects except in the amount of lipotropic factors. When this basal diet was adequately supplemented with choline chloride good growth (slightly better than 3 g. per day) occurred. Further, the diet was designed to avoid an excessive accumulation of fat in the liver, since, as was mentioned earlier, it would be impossible to assess the effects of alcohol upon liver fat if they were superimposed on a liver made excessively fatty by the basal diet. The failure of some investigators to observe this precaution makes it difficult to interpret their data.

We do not agree that Ashworth's findings indicate that alcohol exerts a specific toxic action on liver cells. He reported that rats consuming high-casein diets showed fatty changes in the liver when given alcohol and that pair-fed controls did not. It may be noted that both groups of his rats lost weight. In the control rats this weight loss was probably due to their food intake being restricted to that of the rats given alcohol. It should be noted, however, that part of the weight loss might be explained by the absence from the diet of some of the B vitamins. It is now well known that the requirement for lipotropic agents diminishes as food intake is restricted, whether this restriction is imposed deliberately or whether it results from a deficiency of one or more vitamins. Thus it is not surprising that the livers of his control rats consuming the high-casein diet were not fatty. However, the rats given alcohol received more calories and lost less weight, but obtained no more dietary lipotropic factors than did the pair-fed controls. The increased requirement for lipotropic substances in this group is shown by the fatty changes in the liver which were found in all these animals. Thus, it is unnecessary to invoke any specific effect of alcohol to account for these results. They are presumably due to a relative deficiency of the lipotropic factors.

In the present experiments the character and distribution of the pre-cirrhotic lesion found in the rats ingesting alcohol differed in no way from that seen in the animals which received isocaloric amounts of sugar. Chaikoff and Connor (1940) stated that the diffuse character of the hepatic fibrosis produced in dogs by dietary means differed in type from that which they had previously described (Chaikoff and Connor, 1938) in dogs receiving alcohol. The photomicrographs published by them suggest to us that they may have been comparing different stages of the same type of lesion in their two groups of dogs. We agree with Lowry, Daft, Sebrell, Ashburn, and Lillie (1941) that alcohol aggravates dietary cirrhosis without altering the type or distribution of the fibrous tissue.

The importance of dietary factors in the prevention and treatment of liver cirrhosis has been discussed by many writers in recent years. The views expressed recently by Patek (1947) and Himsworth (1948) are supported and extended by the experimental studies which are here reported.

It is obvious that choline and other essentials should be supplied as naturally occurring components of a well-balanced diet, but the dietary faddist, alcoholic, or "soft-drink" addict does not consume this normal diet. One possibility, therefore, is to add choline or one of its precursors to alcoholic or saccharine* beverages which some individuals use to provide a large part of their calorie intake. As indicated above, the diet of the alcoholic is usually deficient not only in the lipotropic substances but also in other vitamins, and indeed in all essential food factors. The proposed fortification of these beverages with choline (or precursors), while protecting the liver against cirrhosis, would doubtless aggravate lesions due to deficiencies of other vitamins. This suggestion raises scientific, moral, financial, and gustatory problems which are far beyond the scope of this communication. Some preliminary studies of the effect of the lipotropic agents on the taste of various beverages have already been made, but we do not contemplate carrying out a comprehensive investigation of this matter. The necessary researches on the gustatory problems are relatively simple and do not lack interesting features.

Summary and Conclusions

In these experiments on white rats there is no more evidence of a specific toxic effect of pure ethyl alcohol upon liver cells than there is for one due to sugar. This conclusion is based upon the observation that dietary supplements of sucrose cause hepatic lesions (fatty changes and fibrosis) so similar in character and extent to those produced by an isocaloric amount of alcohol that they are indistinguishable.

The results suggest that an imbalance between calorie intake and supply of accessory food factors is the cause of the liver lesions: the extra calorie intake induces a specific deficiency when the basal diet is marginal with respect to any one vitamin. Alcohol or sugar, when taken in excess, supplants choline-containing foodstuffs and at the same time, by increasing the calorie intake, augments the demand for the lipotropic agents.

A mildly hypolipotropic diet is described which allows good growth of young adult male rats and maintains the total liver lipids at about 10% of the fresh tissue weight. This permits the study of the effects of alcohol upon the liver without complications due to multiple dietary deficiencies or to excessive deposition of liver fat resulting from the basal diet alone.

Rats eating this diet *ad libitum* and consuming 15% (v/v) solution of pure ethyl alcohol in place of drinking-water for six months develop fatty livers, and about one-half of them show pre-cirrhotic fibrosis.

An adequate supply of dietary choline or its precursor methionine (either free or in casein) protects the liver; neither excessive deposition of lipids nor fibrosis could be detected in any of the rats receiving lipotropic supplements.

Since the usual vitamins are present in the basal diet in more than adequate amount, and since the lesions are prevented by extra choline, the conclusion may be drawn that the hepatic changes associated with the ingestion of pure ethyl alcohol in this experiment are due to an induced choline deficiency.

*The word used here, "saccharine," it may be unnecessary to note, is the English adjective meaning sweet; it should not be confused with the trade name "Saccharin" applied to the sweet-tasting aromatic compound *o*-sulphobenzimide.

Problems connected with the proposal to supplement alcoholic beverages and saccharine aerated water ("soft drinks") with choline or its precursors are mentioned.

Our findings are, of course, not necessarily applicable to alcoholism in human subjects. If they should prove to be so, it is increasingly obvious that individuals who habitually consume large amounts of alcohol or sugar lack adequate amounts of the lipotropic agents as well as of other dietary essentials.

The classical hepatic lesions (fatty changes and fibrosis) associated with alcoholism in human subjects, the first descriptions of which are mentioned in our introduction, may prove to be due specifically to a lack of the lipotropic agents.

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REFERENCES

Addison, T. (1836). *Guy's Hosp. Rep.*, **1**, 476.
Ashworth, C. T. (1947). *Proc. Soc. exp. Biol., N.Y.*, **66**, 382.
Best, C. H., Lucas, C. C., Patterson, J. M., and Ridout, J. H. (1946). *Biochem. J.*, **40**, 368.
Beveridge, J. M. R., and Lucas, C. C. (1945). *J. biol. Chem.*, **157**, 311.
Chaikoff, I. L., and Connor, C. L. (1940). *Proc. Soc. exp. Biol., N.Y.*, **43**, 638.
—— —— and Biskind, G. R. (1938). *Amer. J. Path.*, **14**, 101.
Connor, C. L., and Chaikoff, I. L. (1938). *Proc. Soc. exp. Biol., N.Y.*, **39**, 356.
Daft, F. S., Sebrell, W. H., and Lillie, R. D. (1941). Ibid., **48**, 228.
Duchek, A. (1853). *Prag. Vjschr.*, **3**, 104. Cited by Ruge (1870).
Himsworth, H. P. (1948). *Sci. Progr.*, **144**, 577.
Huss, Magnus (1852). *Alcoholismus chronicus.* Leipzig and Stockholm. Cited by Ruge (1870).
Kremiansky, J. (1868). *Virchows Arch.*, **42**, 340.
Lillie, R. D., Daft, F. S., and Sebrell, W. H. (1941). *Publ. Hlth Rep., Wash.*, **56**, 1255.
—— Ashburn, L. L., Sebrell, W. H., Daft, F. S., and Lowry, J. V. (1942). Ibid., **57**, 502.
Lowry, J. V., Daft, F. S., Sebrell, W. H., Ashburn, L. L., and Lillie, R. D. (1941). Ibid., **56**, 2216.
—— Ashburn, L. L., Daft, F. S., and Sebrell, W. H. (1942). *Quart. J. Stud. Alcohol*, **3**, 168.
Mitchell, H. H. (1935). *J. Nutrit.*, **10**, 311.
—— and Curzon, E. G. (1940). *Quart. J. Stud. Alcohol*, **1**, 227.
Moon, V. H. (1934). *Arch. Path.*, **18**, 381.
Patek, A. J. (1947). *J. Mt Sinai Hosp.*, **14**, 1.
Rokitansky, C. von (1849). *A Manual of Pathological Anatomy*, **2**, 145. Sydenham Society, London.
Ruge, P. (1870). *Virchows Arch.*, **49**, 252.

THE PRE-ERYTHROCYTIC STAGE OF PLASMODIUM FALCIPARUM

A PRELIMINARY NOTE

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The demonstration of a pre-erythrocytic cycle in mammalian malaria as it exists in the monkey malaria parasite, *Plasmodium cynomolgi* (Shortt and Garnham, 1948), led to the deduction that a similar cycle would be found in the case of human malaria caused by the parasite *P. vivax*. This followed from the close similarity of the two parasites, and the assumption was quickly proved experimentally by the discovery of the pre-erythrocytic cycle in a human volunteer (Shortt, Garnham, Covell, and Shute, 1948). In both cases the pre-erythrocytic cycle was shown to take place in the parenchyma cells of the liver.

It was subsequently demonstrated that this cycle persisted in the liver after the establishment of the infection in the peripheral blood and that it was the probable cause of relapses in benign tertian malaria, since it could exist in the absence of the erythrocytic cycle.

The work of Fairley (1945, 1947) showed conclusively that in both vivax and falciparum malaria there was a cryptic phase in infections caused by mosquito bites during which pre-erythrocytic development must have been proceeding in some situation other than the blood stream. No other conclusion could be drawn from the results of his experiments, and he established the approximate duration of this cryptic phase as eight days in the case of vivax malaria and six days in the case of falciparum malaria.

As already stated above, the truth of this assumption had been established in the case of vivax malaria, but similar proof was still lacking in the case of falciparum malaria, and it was to obtain this that the experiment which is the subject of this paper was carried out.

Unlike the case in vivax malaria, we were unable to argue from monkey to man, since no suitable monkey parasite closely similar to *P. falciparum* was available for study, and a human experiment was necessary without any preliminary comparative work.

The fact that *P. falciparum* shows considerable differences in its morphology and life cycle from *P. vivax* at least raised the possibility that there might be certain differences in the nature of the cryptic stage as compared with those of *P. vivax* and *P. cynomolgi*, as well as in the site in the body where it occurred. It seemed probable, also, if we accept the persisting exo-erythrocytic cycle as the source of relapses in vivax malaria, that, although there would be a pre-erythrocytic cycle in falciparum malaria, it would be short-lived and end with establishment of the erythrocytic infection. This is a natural deduction from the well-known fact, so conclusively proved by Fairley's experiments alluded to above, that complete eradication of the erythrocytic infection in falciparum malaria ends the disease and relapses do not occur. This made the problem of choosing the correct day for the biopsy on the volunteer of great importance, as will be seen when the details of the experiment are described.

Description of Experiment

Anopheline mosquitoes of the species *A. maculipennis atroparvus* and *A. quadrimaculatus* were infected by feeding on two occasions on a subject carrying in his blood large numbers of gametocytes of a Rumanian strain of *P. falciparum*. These were maintained on blood meals for fourteen days, and mosquitoes were dissected and examined daily to follow the progress of the infection. These dissections revealed an infection rate of 93%. After ten days the dissections showed that the salivary glands had been invaded, and it was considered that four days afterwards would be the optimum time to feed them on the volunteer.

This operation was carried out on three days as follows, a total of 770 bites by the mosquitoes being recorded.

On October 10, 1949, 350 mosquitoes fed ; on October 11, 95 fed, these being the mosquitoes that had failed to feed on the first day. On October 12, 325 fed : the majority of these were the mosquitoes of the first day of feeding having a second blood meal on the volunteer.

The dissections referred to had shown that 93% of the mosquitoes were infected and that the infections in the salivary glands were extremely heavy, so that the dosage of sporozoites injected by the 770 bites must have been enormous.

After a full consideration of all the factors involved, and influenced chiefly by the duration of the incubation period